Effective Treatment of Pancreatic Tumors With Two Multimutated Herpes Simplex Oncolytic Viruses

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Pancreatic cancer is an aggressive, rapidly fatal disease against which current nonsurgical therapy has minimal impact. This study evaluates the efficacy of two novel, replication-competent, multimutated herpes viruses (G207 and NV1020) in an experimental model of pancreatic cancer. Four human pancreatic carcinoma cell lines were exposed to G207 or NV1020, and cell survival and viral progeny production were determined. Flank tumors in athymic mice were subjected to single or multiple injections of 1×10^7 G207 or NV1020, and tumor volume was evaluated over time. For all of the cell lines, G207 and NV1020 produced infection, viral replication, and cell lysis ($P < 0.05$). NV1020 resulted in a higher production of viral progeny compared to G207. The efficacy of viral tumor cell kill was greatest in those cells with the shortest in vitro doubling time. For flank tumors derived from hs766t, single or multiple injections of both viruses were equally effective and significantly reduced flank tumor burden ($P < 0.05$). Complete hs766t flank tumor eradication was achieved in 25% (5 of 20) of animals treated with G207 and 40% (8 of 20) of animals treated with NV1020. In vivo efficacy correlated with in vivo tumor doubling time. There were no adverse effects related to viral administration observed in any animal. NV1020 and G207 effectively infect and kill human pancreatic cancer cells in vitro and in vivo. Given the lack of effective nonoperative treatments for pancreatic cancer, oncolytic herpes viruses should be considered for clinical evaluation. (J GASTROINTEST SURG 2000;4:580-588.)

KEY WORDS: G207, NV1020, pancreatic carcinoma, R7020, herpes simplex virus, HSV-1, HSV-2, gene therapy

Pancreatic cancer is an aggressive malignancy with 5-year survival rates of 1% to 4%.[1] It is estimated that there will be 28,300 new cases and 28,200 deaths from pancreatic cancer in 2000.[2] Resection is the only treatment that offers the possibility of long-term survival. Recurrence is common, however, even after complete resection. Currently available therapies have been disappointing in their ability to reduce the incidence of recurrence and have a very limited impact in patients with unresectable disease. Novel treatment strategies are therefore necessary to further reduce the morbidity and mortality of pancreatic cancer.

The use of viruses is central to many antineoplastic strategies that have proved effective in experimental models. Many of these approaches use replication-defective viruses as vectors to transfer genes encoding protein products, such as immunostimulating cytokines,[3,4] prodrugs,[5,6] tumor suppressor genes,[7,8] or other agents. An alternative approach uses replication-competent viruses to infect and lyse tumor cells. In this regard, a number of viruses are currently being examined as treatment for cancer, such as Newcastle disease virus,[9] adenovirus,[10] and herpes simplex viruses.[11-14] A major advantage of replication-competent oncolytic viruses is that they may be administered in relatively small doses because the lytic life cycle generates progeny viruses capable of lysing additional target cells.

Oncolytic herpes viruses were originally examined for the treatment of neurologic tumors, attempting to exploit the natural neurotropism of herpes simplex virus (HSV)[15] and using strains genetically engineered

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to have reduced cytotoxicity to normal tissues and increased specificity toward tumor cells.[15] Initial success spawned further research into the use of these viruses in non-neurologic tumors. Replication-competent oncolytic herpes simplex viruses were subsequently shown to infect and kill diverse cancer types.[11,14,16-19] Two promising viruses in the current generation of replication-competent herpes simplex viruses are G207 and NV1020 (also known as R7020). Both were developed from the strain F backbone of HSV type 1. G207 is a virus designed specifically for cancer therapy that has had both copies of the natural $\gamma_1 34.5$ gene deleted to decrease HSV neurovirulence and the ICP6 gene inactivated to increase the specificity of G207 for rapidly dividing cells.[20,21] NV1020 was initially designed as a vaccine against HSV type 1 and 2 infections and has one copy of the $\gamma_1 34.5$ gene deleted, as well as other gene rearrangements to decrease virulence.[22] The ICP6 gene is intact. This report describes the use of these viruses in the treatment of experimental pancreatic cancer. The data show that both viruses have significant activity against human pancreatic cancer cell lines, both in vitro and in vivo, and that efficacy is greatest in more rapidly dividing cells.

MATERIAL AND METHODS

Viruses

G207 was constructed as previously described.[20] Both copies of the $\gamma_1 34.5$ gene were deleted and the *E. coli* lacZ gene was inserted into the $U_L 39$ sequence to disable production of ribonucleotide reductase.[20] NV1020 was clonally derived from R7020 and constructed as previously described.[22] NV1020 has a 15 kb deletion over the joint region of the HSV-1 genome, which encompasses the genes ICP0, ICP4, and one copy of $\gamma_1 34.5$, as well as latency-associated transcripts. It also has a 700 bp deletion of the endogenous thymidine kinase (TK) locus that prevents expression of the overlapping transcripts of the $U_L 24$ gene. In addition, a 5229 bp fragment of HSV-2 DNA encoding for several glycoprotein genes was inserted into the deleted joint region. An exogenous copy of the HSV-2 TK genes was also inserted, under the control of the $\alpha 4$ promoter. Virus at a multiplicity of infection (MOI) of 0.02 was propagated in African green monkey kidney (VERO) cells obtained from American Type Culture Collection (ATCC, Rockville, Md.) and maintained in Dulbecco's modified Eagle medium and 5% fetal bovine serum. Two days after infection, VERO cells were subjected to freeze-thaw lysis and sonication to release virus. Cell lysates were clarified by centrifugation (300 × *g* for 10 minutes at 4° C), and supernatant fractions containing virus were stored at −80° C. Viral titers were confirmed by plaque assays. Virus was stored at −80° C in NaCl–20 mmol/L Tris at pH 7.5.

Cell Culture

Four human pancreatic cancer cell lines purchased from ATCC were maintained at 37° C in a 5% carbon dioxide humidified atmosphere. HTB147, hs766t (HTB134), and PANC-1 (CRL1469) were maintained in Dulbecco's modified Eagle medium with 4.5 g/L glucose and 10% fetal bovine serum. AsPC (CRL1682) was maintained in RPMI 1640 with sodium pyruvate and 10% fetal bovine serum.

Population Doubling Time

Cells were plated at 5×10^4 cells in replicate T-25 flasks (Costar Corp., Cambridge, Mass.). Over the following 7 days, at 12-hour intervals, monolayers were trypsinized and cells counted on a hemacytometer using trypan blue exclusion. The average number of cells at each time point (n = 2) was plotted as a function of time, and doubling time was extrapolated.

Survival Assay

Viability of the four human pancreatic cancer cell line populations after exposure to G207 or NV1020 was determined as follows: cells were plated at a density of 4×10^4 cells per well in 24-well plates (Costar Corp.). Twelve hours later, medium was aspirated and the appropriate concentration of virus in fresh medium was applied in a final volume of 1 ml. Virus was added at multiplicities of infection (ratio of virus to cells) of 0.01, 0.1, and 1.0. At 1, 2, 3, and 4 days after infection, wells were washed with 0.25% EDTA and exposed to 0.25% trypsin. Cells were counted on a hemacytometer using trypan blue exclusion of nonviable cells. Assays were performed in triplicate. Survival of each cell line population was calculated as the number of cells in treatment groups divided by the number of cells in control (medium only) wells and multiplied by 100%.

Viral Plaque Assays

To demonstrate viral replication, standard plaque assays were performed in duplicate on hs766t and HTB147 cell lysates and supernates. The cell lines chosen for plaque assay had the longest and shortest doubling times, respectively. Cells plated at 5×10^5 cells per well in six-well plates (Costar Corp.) were infected with G207 or NV1020 at MOI 0.01. For each daily time point, cells were scraped from each

well in their medium, transferred to 15 ml polypropylene tubes (Becton Dickinson Labware, Franklin Lakes, N.J.), and subjected to four cycles of freeze-thaw lysis, and centrifugation at 3000 × *g* for 10 minutes at 4° C. Supernatant fractions were added to confluent VERO cells for standard plaque assay. Plaques were counted 3 days after VERO cell infection, and recovered titers were determined. MOI was calculated by dividing recovered titers by the number of viable cells in the survival assay.

Treatment of Flank Tumors in Athymic Mice

All experiments were performed with approval of the Memorial Sloan-Kettering Institutional Animal Care and Use Committee. Animals were housed in pathogen-free quarters in the animal facility, and weighed and examined three times per week.

Separate experiments with hs766t and HTB147 human pancreatic cancer cells were performed identically. The cell lines chosen for the in vivo study had the longest and shortest doubling times, respectively. Twenty-five athymic mice (National Cancer Institute), aged 4 to 6 weeks, were anesthetized with methoxyflurane inhalation; 2×10^6 cells of the pancreatic cancer cell line were injected subcutaneously in serum-free medium bilaterally on the flank. Tumors were measured three times per week. Tumor volume was calculated using the formula $4/3\,\pi ab^2$, where "a" is the radius of the long axis and "b" the radius of the short axis in millimeters. When tumor volume reached 22 ± 3 and 30 ± 3 mm^3 (± SEM) for hs766t and HTB147, respectively, animals were sorted into five treatment groups of n = 10 tumors for injection of agents in a volume of 0.075 ml serum-free medium as follows: single dose of 1×10^7 plaque-forming units (pfu) G207, three daily doses of 1×10^7 pfu G207, single dose of 1×10^7 pfu NV1020, three daily doses of 1×10^7 pfu NV1020, or three daily doses of serum-free medium. Duration of response was monitored for 3 weeks. Control medium, both in vitro and in vivo, was either serum-free medium or phosphate-buffered saline solution. Previous studies in our laboratory showed no difference between the heat-inactivated virus and culture media or phosphate-buffered saline solution alone.

Statistical Analysis

Student's two-tailed *t* test was used to determine significance between various treatment groups in both the in vitro and in vivo experiments.

RESULTS

Survival Assay

Survival of the cell lines after infection with G207 or NV1020 decreased compared to control specimens after 1 day of treatment (Student's *t* test, $P < 0.05$) for all MOIs tested (Fig. 1). A virus-to-cell ratio (MOI) of 1 was more effective in killing cells than MOIs 0.1 and 0.01 for all cell lines ($P < 0.05$). At 4 days, HTB147 and AsPC infected with G207 or NV1020 at a MOI of 1 demonstrated less than 5% survival (Table I). Cell survival was lowest in those cell lines with the shortest in vitro doubling times. NV1020 was more effective than G207 at killing AsPC cells after 2 days at all MOIs ($P < 0.01$). Doubling times of the four human pancreatic cancer cell lines examined are listed in Table I. The most rapidly dividing was HTB147 (30 hours) and the most slowly dividing was hs766t (72 hours). Tumor doubling time correlated with survival after infection with G207 or NV1020.

Viral Plaque Assays

Viral progeny production was quantified after infection with G207 or NV1020 in hs766t and HTB147, the cell lines with the longest and shortest doubling times, respectively. Both cell lines supported G207 and NV1020 viral proliferation (Fig. 2). From the initial MOI of 0.01, the ratio of virus to cells increased

Table I. Survival of human pancreatic cancer cell lines after 4 days of exposure to G207 or NV1020

Cell line	Histology	TD (hr)*	G207†			NV1020		
			MOI 0.01	MOI 0.1	MOI 1	MOI 0.01	MOI 0.1	MOI 1
HTB147	Adenocarcinoma	30	53 (±2)	31 (±3)	2 (±2)	32 (±12)	23 (±5)	3 (±2)
AsPC	Adenocarcinoma	48	85 (±23)	42 (±20)	4 (±1)	38 (±14)	5 (±3)	1 (±2)
PANC-1	Epithelioid carcinoma	57	71 (±14)	51 (±20)	27 (±2)	73 (±9)	64 (±3)	28 (±3)
hs766t	Adenocarcinoma	72	79 (±9)	71 (±8)	26 (±5)	71 (±13)	41 (±14)	23 (±7)

MOI = multiplicity of infection, which is the ratio of virus to cells.
*Cell line population doubling time measured in hours.
†Results are percentages ± standard deviation. All assays were performed in triplicate. Cells exposed to virus and cells in serum-free media were counted on a hemacytometer using trypan blue exclusion of nonviable cells. Survival of each cell line population was calculated as the average (n = 3) number of cells in treatment groups divided by the average (n = 3) number of cells in the control (medium only) wells.

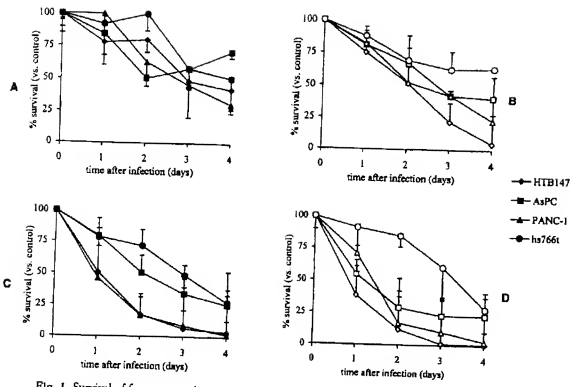

Fig. 1. Survival of four pancreatic cancer cell lines after exposure to G207 or NV1020. Survival assays were performed in triplicate on AsPC, hs766t, HTB147, and PANC-1. Cells were infected with G207 (black symbols) or NV1020 (white symbols) at MOIs of 0.01, 0.1, or 1, and were monitored each day for viability using trypan blue exclusion. Cell survival for each cell line was calculated by dividing viability in each treatment group by the viability in uninfected control wells and multiplying by 100%. Cell survival is plotted as a function of time for G207, MOI 0.1 (A); NV1020, MOI 0.1 (B); G207, MOI 1 (C); and NV1020, MOI 1 (D). MOI 0.01 is not shown. Survival of all cell lines after infection with G207 or NV1020 decreased compared to control values after 1 day of treatment (Student's t test, $P < 0.05$). MOI 1 was more effective at cell killing than MOIs of 0.1 and 0.01 in each cell line ($P < 0.05$). NV1020 was more efficient than G207 after 2 days in killing AsPC cells at all MOIs ($P < 0.01$).

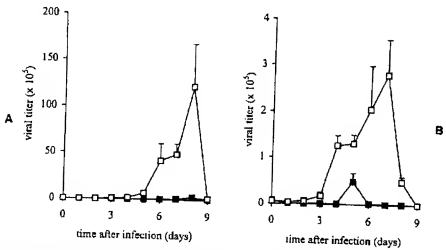

Fig. 2. Replication of G207 and NV1020 in hs766t (A) and HTB147 (B) pancreatic cancer cell lines after infection at MOI 0.01. Cell lysates and supernates from duplicate treatment wells were collected every 24 hours and titrated by standard plaque assay. Viral titer is plotted as a function of time. The peak viral titer was higher for NV1020 (white squares) than G207 (black squares) in both cell lines, by a factor ranging from 43 for hs766t to 6 for HTB147. From an initial MOI of 0.01 (5000 pfu), the ratio of G207 virus to cells increased 58 times for hs766t and 10 for HTB147. For NV1020, the maximum MOI increased 2444 and 56 times, respectively.

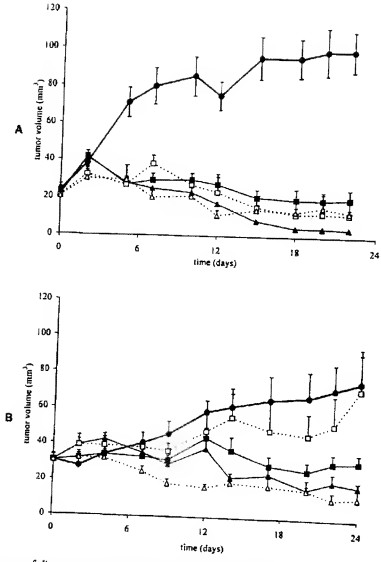

Fig. 3. Efficacy of direct intratumoral G207 or NV1020 injection in the treatment of hs766t (A) and HTB147 (B) pancreatic cancer flank tumors. At treatment, pancreatic cancer tumor volumes in athymic mice were 22 ± 3 mm³ and 30 ± 3 mm³ (± standard error of the mean) for hs766t and HTB147, respectively. Tumors were directly injected, in a volume of 0.075 ml, with either three doses of serum-free medium administered over 5 days (control, black circles), 1×10^7 pfu of G207 (G207 single-dose, black boxes), three doses of 1×10^7 pfu of G207 administered over 5 days (G207 three doses, black triangles), 1×10^7 pfu of NV1020 (NV1020 single-dose, white squares), or three doses of 1×10^7 pfu of NV1020 administered over 5 days (NV1020 three doses, white triangles). Duration of response was monitored for 3 weeks. Average tumor volume (± standard error of the mean) is plotted as a function of time. (A), hs766t tumor volume in all treatment groups was decreased compared to control values ($P < 0.0002$). Tumors in the G207 three dose treatment groups were smaller than those in the respective single-dose treatment groups ($P < 0.02$). (B), HTB147 tumor volume in the G207 treatment groups and the NV1020 three-dose group was decreased compared to control values ($P < 0.05$). Tumors in both three-dose treatment groups were smaller than those in their respective single-dose treatment groups ($P < 0.04$).

Table II. Mean tumor volume and proportion of nonpalpable tumor for each treatment group

Treatment group*	Mean hs766t volume†	Proportion with no hs766t tumor‡	Mean HTB147 volume†	Proportion with no HTB147 tumor‡
Control	99 ± 11	0/10	76 ± 16	0/10
G207 single-dose	20 ± 4§	1/10	32 ± 5§	0/10
G207 three doses	4 ± 2§	4/10	19 ± 3§	0/10
NV1020 single-dose	12 ± 3§	4/10	72 ± 23	0/10
NV1020 three doses	13 ± 6§	4/10	12 ± 5§	0/10

*hs766t tumors of 22 ± 3 mm³ and HTB147 of 30 ± 3 mm³ (± standard error of the mean) in athymic mice were directly injected with serum-free medium (control), 1×10^3 pfu of G207 (G207 single-dose), 1×10^7 pfu of NV1020 (NV1020 single-dose), three doses of 1×10^7 pfu of G207 administered over 5 days (G207 three doses) or three doses of 1×10^7 pfu of NV1020 administered over 5 days (NV1020 three doses). Duration of response was monitored for 3 weeks.

†Results are mean (n = 10 tumors per treatment group) tumor volume ± standard error of the mean.

‡Results are number of animals with nonpalpable tumors at the end of the experiment.

§Tumor volume in all treatment groups was decreased compared to control values ($P < 0.0002$ for hs766t, $P < 0.04$ for HTB147). For hs766t tumors, tumors in the G207 three-dose treatment groups were smaller than in the respective single-dose treatment group ($P < 0.02$). For HTB147 tumors, tumors in the G207 three-dose treatment groups and the NV1020 treatment groups were smaller than in the respective single-dose treatment groups ($P < 0.02$ and $P < 0.04$, respectively).

10 times for HTB147 and 58 times for hs766t. For NV1020, maximum MOIs increased 56 and 2444 times, respectively. In both cell lines, peak viral titer was higher for NV1020 than G207 by a factor ranging from 6 for HTB147 to 43 for hs766t.

Treatment of Flank Tumors in Athymic Rats and Mice

Efficacy of intratumoral viral injection in suppressing flank xenografts in athymic mice was measured. HTB147 and hs766t were chosen for the experiment because they had the shortest and longest in vitro doubling times, respectively. It should be noted that, although HTB147 had the shortest in vitro doubling time, it grew slowly in vivo, with a doubling time of 10 days, compared with 3 days for hs766t. At the end of treatment, average hs766t tumor volumes were as follows: control, 99 ± 11; G207 single-dose, 20 ± 5; NV1020 single-dose, 12 ± 3; G207 three doses, 4 ± 2; and NV1020 three doses, 13 ± 6 mm³ (Fig. 3, *A*). The average HTB147 tumor volumes were as follows: control, 76 ± 16; G207 single-dose, 32 ± 5; NV1020 single-dose, 72 ± 23; G207 three doses, 19 ± 3; and NV1020 three doses, 12 ± 5 mm³ (Fig. 3, *B*). Starting at 5 days (hs766t) and 9 days (HTB147) after treatment, tumor volume was lower in all treatment groups compared to control values ($P < 0.01$), with the exception of HTB147 tumors treated with NV1020 single-dose, which did not differ in size significantly from control values. Twenty-five percent (5 of 20) of hs766t tumors treated with G207 and 40% (8 of 20) treated with NV1020 were nonpalpable at 22 days after treatment (Table II). There was no complete response to viral treatment in HTB147 tumors. For hs766t, G207 three doses was more effective than G207 single-dose ($P < 0.02$), whereas both modes of NV1020 administration were equally effective (see Fig. 3, *A*). For HTB147, three-dose administration of each virus was more effective than the respective single-dose treatment ($P < 0.04$).

DISCUSSION

In order for a replication-competent oncolytic virus to be effective for cancer therapy, it must be able to infect the tumor cell of interest, replicate to produce progeny, and lyse the cells. This efficacy must then be balanced against the safety profile of the agent. G207 has been shown to be effective in a variety of malignancies, including glioma,[12] mesothelioma,[23] head and neck[16] and prostate[24] cancer cells, and metastases to the brain,[25] liver,[17] and peritoneum.[19] The efficacy of NV1020 has been described previously in head and neck cancers.[26] In pancreatic cancer cells the oncolytic properties of G207 have been described in vitro.[18] The current experiments extend these observations to another promising virus and also examine both viruses in vivo. The present study demonstrates that G207 and NV1020 effectively infect, replicate within, and kill human pancreatic cancer cells both in vitro and in vivo.

The results of these experiments suggest that the proliferate characteristics of the target cell predict the response to viral treatment. The relatively more rapidly growing pancreatic cancer cell lines AsPC and HTB147 had a greater response to both viruses than PANC-1 and hs766t in vitro. In other cell lines investigated in our laboratory, the efficacy of G207 also correlated closely with doubling time or S-phase frac-

tion.[16,17,19] However, the in vivo characteristics of tumors may be the most important determinants of clinical response to viral therapy. Although hs766t had a longer in vitro doubling time than HTB147, in vivo hs766t was the faster growing tumor and was more efficiently eradicated by both viruses compared to HTB147. The markedly higher viral proliferation in hs766t may account for the high number of complete responses seen with this cell line.

NV1020 produced higher quantities of viral progeny than G207. The presence of one functioning copy of the neurovirulence and replication efficiency $\gamma_1 34.5$ gene in NV1020, which apparently reduces dependence on the host cell for proliferation, may be responsible for this difference in viral growth.[18,28] In the hs766t cell line, NV1020 produced much higher viral titers than G207, but no difference was seen in the ability of each virus to kill hs766t tumors in vivo. This may be due to the efficient killing of hs766t tumors by both viruses. It may be that for a single sensitive cell line, maximum efficacy is achieved at a concentration of virus well below the amount achieved at peak production. Whether NV1020 is more efficient in humans, where tumors are more heterogeneous, requires further clinical investigation.

Since these viruses are replication competent, safety issues relating to viral dissemination and its impact in the host must be addressed. Both viruses were well tolerated by all animals. No animal inoculated with either virus showed morbidity or mortality from the virus, and they continued to eat and groom. All animals survived the experiment. Viral dissemination in normal murine tissues has been quantified previously by our laboratory using real-time quantitative polymerase chain reaction, and no evidence of viral proliferation has been observed in any organ after subcutaneous inoculation of virus.[17] In addition, the multiple mutations within the genome of these viruses make reversion to wild type unlikely. Both viruses retain the thymidine kinase gene, making them sensitive to acyclovir therapy if dissemination occurs. Furthermore, both viruses have been tested in nonhuman primates and have proved to be attenuated compared to wild-type strains.[29-31]

This study has several implications for potential clinical application of oncolytic HSV. In the adjuvant setting, when there may be viable tumor cells present in the resection bed but the tumor cell burden is low, delivery of sufficient virus to reach MOI of 1 to 100 would be feasible. Even with no further local proliferation of virus, such high effective doses of virus will likely kill residual tumor cells. This may be an ideal setting for the use of the more attenuated G207. On the other hand, for patients with large unresectable tumors, and for palliation, it will be important for local production of virus to occur so that progeny virus can enhance antitumor efficacy. In such situations, administration of NV1020 may be preferred, since its relatively higher proliferative rate may result in a higher effective dose at the tumor site.

CONCLUSION

Attenuated, replication-competent herpes simplex viruses G207 and NV1020 are effective at treating experimental human pancreatic cancer by intratumoral injection. Cell survival decreased after infection with each virus compared to control values, and both viruses were able to produce a burst of viral progeny that ranged from 10 to 2444 times the initial MOI. NV1020 had between 6 and 43 times higher viral progeny production than G207. Both viruses were effective at treating pancreatic cancer xenografts, particularly with a multiple-dose regimen. These viruses represent a new class of agents and a novel treatment approach to pancreatic cancer, which is largely resistant to standard therapy.

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Discussion

Dr. Douglas L. Fraker (Philadelphia, Pa.). Regarding neurotropism, are there data in primate models that are a more typical host of these herpes viruses or data that this totally eliminates the neurotoxicity? Could you speculate on how you would get around the problems of delivery in clinical pancreatic cancer, as well as on generation of host immunity?

Dr. Jarnagin. In preclinical toxicology studies, performed in Aotus monkeys that are exquisitely sensitive to wild-type herpes virus, the animals did not develop significant clinical neurologic toxicity. In previous experiments, when we used intraportal injections in a metastatic colon cancer model and then analyzed brain and other tissues by polymerase chain reaction, none of the tissues other than liver had evidence of viral gene presence. As far as delivery is concerned, it depends on the situation. For example, after tumor resection, viruses could conceivably be delivered through an intraperitoneal port or simply placed in the operative field. In a patient with an unresectable tumor, it would be a bit difficult to deliver viruses intra-arterially, but perhaps direct injection even endoscopically would be possible. In regard to host immunity, preformed antibodies to herpes viruses do not eliminate the ability of the viruses to kill cells in primate or mouse models.

Dr. John P. Hoffman (Philadelphia, Pa.). You implied from your introduction that we need better systemic therapies for pancreatic cancer. Have you used this metastatic model to determine whether or not the virus can go elsewhere?

Dr. Jarnagin. Not in pancreatic cancer, but in other models, yes we have. In colorectal cancer cell lines metastatic to the liver, we and others have shown that this is potentially effective, and perfusion of the portal vein with virus actually results in selective infection of the metastatic deposits within the liver.

Dr. S. Archer (Boston, Mass.). Do you have any idea what the mechanism of cytotoxicity is?

Dr. Jarnagin. It seems, looking at the histology, that it is mostly direct cell lysis. There is some evidence of apoptosis, although I do not know if that has been fully resolved.

Dr. Archer. Can you actually hook up a therapeutic transgene to these viruses to affect cell kill?

Dr. Jarnagin. There are now newer viral strains that are replication competent, oncolytic, and express high levels of various stimulatory genes, such as IL-12 and IL-2.